

# Factors Promoting Acute and Chronic Diseases Caused by *Yersinia*

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## INTRODUCTION

Mankind has been sorely vexed during this nearly finished millennium by the consequences of both acute epidemic diseases (including relapsing and typhoid fevers, cholera, and typhus, but now no longer smallpox virus) and severe chronic afflictions (e.g., tuberculosis, leprosy, syphilis, malaria, and now human immunodeficiency virus). However, morbidity and mortality caused by these infections pale in comparison to the devastation wrought by the pestilence, black death, or bubonic plague caused by *Yersinia pestis*. Credible estimates of the number of people killed by this bacterium during the course of history approach 200 million. The organism is known to be lethal to other primates and most rodents often at infecting doses of 10 cells or less (29, 116). The purpose of this review is to discuss recent progress made in understanding the biochemical mechanisms used by *Y. pestis* to execute its extraordinary host range and lethality.

To achieve this objective, the regulation, structure, and function of known and putative virulence factors will be defined and equated when possible to the pathology of experimental infection. Emphasis will be placed on metabolic restrictions that relegate *Y. pestis* to a protected life cycle comprising its flea vector and rodent host. Expression of striking lethality as a consequence of this adaptation will

be discussed with reference to chronic disease promoted by closely related enteropathogenic *Y. pseudotuberculosis* and *Y. enterocolitica*.

## GENERAL PROPERTIES

The yersiniae (genus XI of the family *Enterobacteriaceae*) consist of seven species of which *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* are considered to be primary pathogens of mammals; only these medically significant yersiniae are considered in this review. Wild-type *Y. pestis* and *Y. pseudotuberculosis* exhibit nearly identical chromosomal DNA relatedness (10, 19, 103); distinctions mainly represent differences in plasmid content (8, 59), as discussed below. Chromosomal DNA of *Y. enterocolitica* possesses significantly less relatedness, indicating that the advent of its evolutionary divergence occurred prior to that separating *Y. pseudotuberculosis* and *Y. pestis* (10, 19, 103).

## Physiology

The medically significant yersiniae can multiply on appropriate media at temperatures ranging from about 5 to 42°C. However, marked differences mediated by global regulatory mechanisms occur upon an increase from room (26°C) to host (37°C) temperature. These dysfunctions include expression of additional nutritional requirements and production of virulence functions. For example, the enteropathogenic yersiniae can form visible colonies at 26°C in 24 to 36 h on

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solid medium containing essential inorganic salts and a fermentable carbohydrate. Similar growth at 37°C, however, typically requires addition of the aspartic acid family of amino acids and is often enhanced by the vitamins thiamine and pantothenate (21, 22). In contrast, *Y. pestis* exhibits an obligatory nutritional requirement at all permissible temperatures for L-methionine, L-phenylalanine, L-isoleucine, L-valine, and glycine (or L-threonine); growth of this organism at 37°C is also markedly improved by additional amino acids and vitamins (26, 31, 74). Formation of colonies by *Y. pestis* at 26°C on minimal or at 37°C on enriched solid medium takes about 48 h as opposed to 18 to 24 h for enteropathogenic yersiniae. This period can also be shortened to 24 h for *Y. pestis* when the organisms are incubated in a flowing 5% CO<sub>2</sub> incubator (21).

Comparative studies of catabolic mechanisms indicate that the enteropathogenic yersiniae possess intact heterotrophic pathways typical of members of the *Enterobacteriaceae*. However, some striking enzyme omissions occur in *Y. pestis* which result in inefficient metabolism of small molecules in most in vitro environments. This organism lacks detectable glucose 6-phosphate dehydrogenase and thus, being unable to use the hexose monophosphate pathway, must synthesize pentose via rearrangements catalyzed by transketolase and transaldolase (105). *Y. pestis* may also lack a completely functional tricarboxylic acid cycle, as judged by its possession of a unique constitutive glyoxylate bypass (76) and a possible lesion at the level of  $\alpha$ -ketoglutarate dehydrogenase (21). The absence of aspartase (preventing conversion of aspartate to fumarate) promotes a linked defect that blocks generation of oxalacetate via fumarase and malate dehydrogenase. The oxalacetate pool is further reduced by transamination to inert aspartate, which limits its conversion to catabolically useful citrate. As a consequence, aspartate accumulates as a metabolic byproduct in *Y. pestis*, whereas this amino acid undergoes rapid catabolism in *Y. pseudotuberculosis* (52). The absence of aspartase in *Y. pestis* may account for the stimulatory effect of CO<sub>2</sub> on growth by facilitation of phosphoenolpyruvate carboxylation (6), thereby replenishing the oxalacetate pool.

*Y. pestis* lacks additional enzymes present in *Y. pseudotuberculosis*, which are less central to intermediary metabolism. Their absence, however, is diagnostic; they include urease and activities that catalyze synthesis of the nutritionally required amino acids, initial catabolism of rhamnose and melibiose, and assimilation of low levels of ammonia (26, 56, 57). In addition, *Y. pestis* but not *Y. pseudotuberculosis* promotes extremely rapid catabolism of L-serine (52) due to evidently unregulated expression of L-serine deaminase activity (21). This intense reaction may account for the novel nutritional requirement for glycine, a known product of serine anabolism. These physiological distinctions between *Y. pestis* and the enteropathogenic yersiniae are listed in Table 1.

#### Regulation of Macromolecular Synthesis

Mechanisms concerned with the assemblage of macromolecules are typically conserved; thus, unique biosynthetic reactions in yersiniae are unanticipated and have not yet been reported. However, the organisms possess novel regulatory functions that respond to temperature and to the presence of Ca<sup>2+</sup>. These mechanisms plus a typical Fur protein-mediated response to iron privation are involved in expression of virulence and are discussed below. *Y. pestis* is

capable of a normal stringent response (38) upon starvation for an essential amino acid (40). Nevertheless, in comparison to the enteropathogenic yersiniae, cells of this species often exhibit a sluggish recovery following nutritional or temperature shiftup. It is therefore advisable in studies of macromolecular synthesis to grow the organisms for at least one subculture within a new environment to assure existence of the steady state.

#### LPS

Lipopolysaccharide (LPS) of *Y. pestis*, although extractable in hot phenol-water (92), is rough in the sense that it possesses core components but lacks extended O-group structure (18, 117). LPS of *Y. pseudotuberculosis* grown at 37°C is similarly rough, whereas that isolated after incubation at 26°C possesses short O-group side chains containing the hydrophobic serodominant 3,6-dideoxyhexoses abequose, ascarylose, colitose, paratose, and tyvelose (50, 131). *Y. enterocolitica* exhibits a more typical enteric LPS structure containing long O-group side chains which, however, may also be enriched or exclusively composed of hydrophobic sugars (1, 77, 117). Endotoxicity of LPS from *Y. pestis* (2) and probably that of the enteropathogenic yersiniae are comparable to preparations from other enteric species. LPS structure accounts in part for resistance to the antibacterial activity of serum (117).

#### Genetics

Although yersiniae lack known intrinsic vectors of conjugation or transduction (166), sex factors (88) or certain bacteriophages (87, 118) of *Escherichia coli* can promote limited gene exchange. Similarly, modern methods of recombinant technology developed with *E. coli* (93) are almost always applicable to yersiniae. The use of transposon (16, 118, 159, 161) or bacteriophage (68, 113, 114, 144, 147) fusion mutagenesis proved to be especially effective provided that the potential polarity of these inserts was recognized and that precautions were taken to ensure their stability. Transfer of virulence plasmids or cloning vectors is easily accomplished by electroporesis (42, 114).

The concept of meiotrophy or the ability to gain a phenotypic determinant by mutation (56) is an interesting aspect of yersinia genetics. In this situation, *Y. pestis* gains the new property, which is normally expressed as a typical characteristic of wild-type *Y. pseudotuberculosis*. The first such example to be described was acquisition of ability to ferment rhamnose, a mutation that occurred at the rate of 10<sup>-13</sup> (57). Results of later studies showed that many additional diagnostic determinants of *Y. pseudotuberculosis* could also be expressed by meiotrophic mutants of *Y. pestis* (Table 1). This phenomenon indicates that the corresponding genes have been retained within the chromosome in a cryptic state. The nature of the meiotrophic events permitting normal expression of these genes is unknown. Perhaps the lesion preventing their expression is analogous to the single base deletion preventing production in *Y. pestis* of the *yadA* product (128), a plasmid-encoded adhesin of enteropathogenic yersiniae noted below.

#### ENVIRONMENTAL STRICTURES

*Acanthamoeba castellanii*, histotoxic clostridia, and *Pseudomonas pseudomallei* are among a small group of organisms capable of maintaining their population indefinitely in

TABLE 1. Distinguishing properties and virulence determinants of wild-type *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*

Determinant	Established or putative virulence function	<i>Y. pestis</i>	<i>Y. pseudotuberculosis</i>	<i>Y. enterocolitica</i>
ca. 10-kb Pst plasmid		(Present)	(Absent)	(Absent)
Pesticin	0	+	0	0
Plasminogen activator	+	+	0	0
Posttranslational degradation of Yops <sup>a</sup>	+	+	0	0
ca. 70-kb Lcr plasmid		(Present)	(Present)	(Present)
Yops	+	+	+	+
YadA (protein 1 or Yop A)	+	0 <sup>a</sup>	+	+
V, W antigens	+	+	+	+
ca. 100-kb Tox plasmid		(Present)	(Absent)	(Absent)
Fraction 1 or capsular antigen	+	+	0	0
Murine exotoxin	+	+	0	0
Chromosomal				
Pigmentation or peptide F (hemin storage at 26°C)	+	+	0	0
Motility (26°C)	0	0	+	+
Hydrophobic sugars in LPS (26°C)	0	0	+	+
Assimilation of low levels of NH <sub>4</sub> <sup>+</sup> (26°C)	0	0 <sup>b</sup>	+	+
Constitutive glyoxylate bypass	0	+	0	0
Aspartase	0	0	+	+
Glucose 6-phosphate dehydrogenase	0	0	+	+
Urease	0	0 <sup>b</sup>	+	+
Ornithine decarboxylase	0	0	0	+
Host cell invasins				
inv product	+	0 <sup>b</sup>	+	+
ail product	+	0	0	+
Antigen 4 (pH 6 antigen)	+	+	+	0
Antigen 5 (catalase)	+	+	+	0
Fermentation of				
Rhamnose	0	0 <sup>b</sup>	+	0
Melibiose	0	0 <sup>b</sup>	+	0
Sucrose	0	0	0	+
Sorbitol	0	0	0	+
Cellobiose	0	0	0	0
Biosynthesis of				
Methionine	0	0 <sup>b</sup>	+	+
Phenylalanine	0	0 <sup>b</sup>	+	+
Threonine-glycine	0	0 <sup>b</sup>	+	+
Isoleucine-valine	0	0 <sup>b</sup>	+	+

<sup>a</sup> Process mediated by plasminogen activator causing turnover of the outer membrane surface, thereby preventing opsonization.<sup>b</sup> Functions capable of undergoing meiotrophic reversion or known to be encoded by cryptic genes.

soil and water while also possessing the ability to cause severe systemic disease. More typically, the parasitic habit is obligatory due to the absence of functions required for successful prolonged competition with the normal flora of natural environments (106). Most pathogenic organisms, especially those transmitted by the respiratory route or by sexual contact, would thus become rapidly eliminated in nature following separation from their hosts. However, gastrointestinal parasites are typically more sturdy and can often survive without net increase for prolonged periods of time in habitats contested by saprophytes. The enteropathogenic yersiniae are excellent examples of this group, whereas *Y. pestis* is more fastidious and has evolved a distinct strategy of survival. As a consequence, this species

differs markedly from the enteropathogenic yersiniae with respect to phenotype and virulence.

### Ecology

As noted previously *Y. pseudotuberculosis* and *Y. enterocolitica* are robust organisms capable of long-term survival in natural environments due, in part, to their minimal nutritional requirements and ability to remain metabolically active at extremes of temperature. This capacity to remain viable in nature for extended periods of time as a fecal contaminant is, of course, especially advantageous to these organisms, which are transmitted to hosts via the oral route. In contrast, the organic nutritional requirements and slug-

gish regulatory responses of *Y. pestis* serve to reduce its ability to exist independently in nature. Of course, this limitation is meaningless because the plague bacillus is typically maintained in natural environments within the stomach and proventriculus of its flea vector. Little is known about the composition of this unique niche except that it originates as a blood meal from a bacteremic host, permits significant multiplication of *Y. pestis* (and thus contains essential amino acids), and is inhibitory to almost all other tested procaryotes (116).

It thus seems likely that, once *Y. pestis* evolved its unique ability to exploit this fixed and protected environment, selective pressure to maintain functions required for prolonged survival in soil and water became relaxed. As a consequence of evident independent random mutational events, *Y. pestis* has now lost the potential to manifest a number of properties expressed by the enteropathogenic yersiniae which undoubtedly favor survival of the latter in soil and water (Table 1).

### Disease

A fundamental tenet of epidemiology is that successful parasites tend to minimize pathology, thereby preserving the host as an essential niche required for their net multiplication and subsequent dissemination (138). This principle holds nicely for the enteropathogenic yersiniae, which generally cause chronic gastrointestinal disease in natural hosts. As a consequence, the organisms are excreted for prolonged periods of time, thereby favoring their ingestion by new hosts.

The same precept fails in the case of *Y. pestis*, which usually kills its host. This exception, however, is understandable upon recollection that bubonic plague is typically acquired via subcutaneous or intradermal injection by flea-bite. That is, with evolution in *Y. pestis* of the ability to survive and grow within the gut of the flea (accompanied by concomitant loss of fitness in natural environments), subsequent selective pressure favored development of mechanisms promoting invasion of tissues, bacteremia, and lethality. Acquisition of the ability to disseminate from peripheral sites of infection was necessary to assure access to favored sites of replication in regional lymph nodes and viscera. Eventual spillage of the organisms into the vascular system from these sites assures their ingestion by the vector, and lethality, which typically follows the onset of bacteremia, serves to prompt the infected flea to depart and search for a new host.

Accordingly, bubonic plague is manifested as an acute, intransigent, and lethal disease since the infective agent requires the death of its host in order to assure perpetuation via transfer by the disenfranchised vector. This strategy is dependent upon mounting an immediate overwhelming attack on the host before its immune system becomes capable of providing significant defense. The traditional tactic of minimizing damage to the host, thereby preserving an essential niche while prolonging dispersal, is thus unproductive for *Y. pestis* although, as already noted, this stratagem is effective for the enteropathogenic yersiniae. As a consequence, yersiniae provide an excellent model for the study of comparative pathogenesis. On the one hand, the system contains the enteropathogenic species which specialize in promoting chronic disease in natural hosts. On the other, it includes *Y. pestis*, which causes perhaps the most severe acute bacterial disease known to humans. (It is interesting that *Bacillus anthracis*, a leading competitor for this dubious

TABLE 2. Lethality of wild-type *Y. pestis* KIM, *Y. pseudotuberculosis* PB1, and *Y. enterocolitica* WA in mice by various routes of injection (21, 24, 29, 36, 37, 151)

Organism	50% lethal dose <sup>a</sup>				
	i.v.	i.p.	s.c.	i.d.	p.o.
<i>Y. pestis</i>	<10	<10	<10	<10	<10 <sup>b</sup>
<i>Y. pseudotuberculosis</i>	ca. 10	ca. 10 <sup>4</sup>	ca. 10 <sup>5</sup>	>10 <sup>7</sup>	ca. 10 <sup>2</sup>
<i>Y. enterocolitica</i>	ca. 10 <sup>2</sup>	ca. 10 <sup>2</sup>	10 <sup>3</sup>	ca. 10 <sup>6</sup>	ca. 10 <sup>2</sup>

<sup>a</sup> i.v., intravenous; i.p., intraperitoneal; s.c., subcutaneous; i.d., intradermal; p.o., per os.

<sup>b</sup> Tissue invasion of *Y. pestis* probably reflects the same mechanisms used for dissemination from other peripheral routes rather than specific processes used by enteropathogenic yersiniae for penetration of the gastrointestinal surface.

distinction, must also kill its host in order to assure sporulation, a requisite for dissemination and subsequent infection.) Since the medically significant yersiniae are closely related, it follows that the few differences known to occur between them (Table 1) are likely to directly mediate the two distinct forms of infection. The remainder of this review is concerned with the nature of these differences.

### PATHOGENESIS

Wild-type strains of *Y. pestis* exhibit a consistent pattern of virulence in both natural and experimental hosts regardless of their biotype or geographic origin. A similarly consistent but distinct pattern, typified by reduced infectivity by peripheral routes of injection (24), is also characteristic of isolates of all serotypes of *Y. pseudotuberculosis*. However, only serotype O:8 strains of *Y. enterocolitica* are capable of causing comparable invasive disease (36). These isolates are commonly encountered in certain cooler rural regions of northeast North America where the species was discovered (132); the epidemiology of this serotype is not yet fully resolved.

Lethality of wild-type *Y. pestis*, *Y. pseudotuberculosis*, and serotype O:8 cells of *Y. enterocolitica* in the white mouse by route of injection is shown in Table 2. The ability of *Y. pestis* to cause death from peripheral sites of infection is unique and an important characteristic of the species. Since virulence via intravenous injection is uniformly high for all three species, this route is commonly used in studies of pathogenesis.

### Kinetics of Growth

*Y. pestis* and the enteropathogenic yersiniae exhibit similar patterns of multiplication after intravenous injection in the white mouse (Fig. 1). The organisms are rapidly removed from the vascular system of this artificial host but emerge within liver, spleen, and lungs, where they undergo linear growth until populations of about 10<sup>6</sup> cells per g of tissue are achieved. Bacteria again appear within the circulation at this time, presumably due to release from exploited niches in organs and destruction or saturation of fixed macrophages necessary for filtration of blood (135, 147, 151, 152). The apparent rate of growth in organs decreases thereafter due to spillover, causing pronounced bacteremia, an ominous symptom of disease caused by all yersiniae (32, 151). Terminal populations in organs of mice infected with enteropathogenic yersiniae approximate 10<sup>8</sup> to 10<sup>10</sup> cells per g, whereas those following infection with *Y. pestis* are significantly less. This enhanced virulence was attributed to

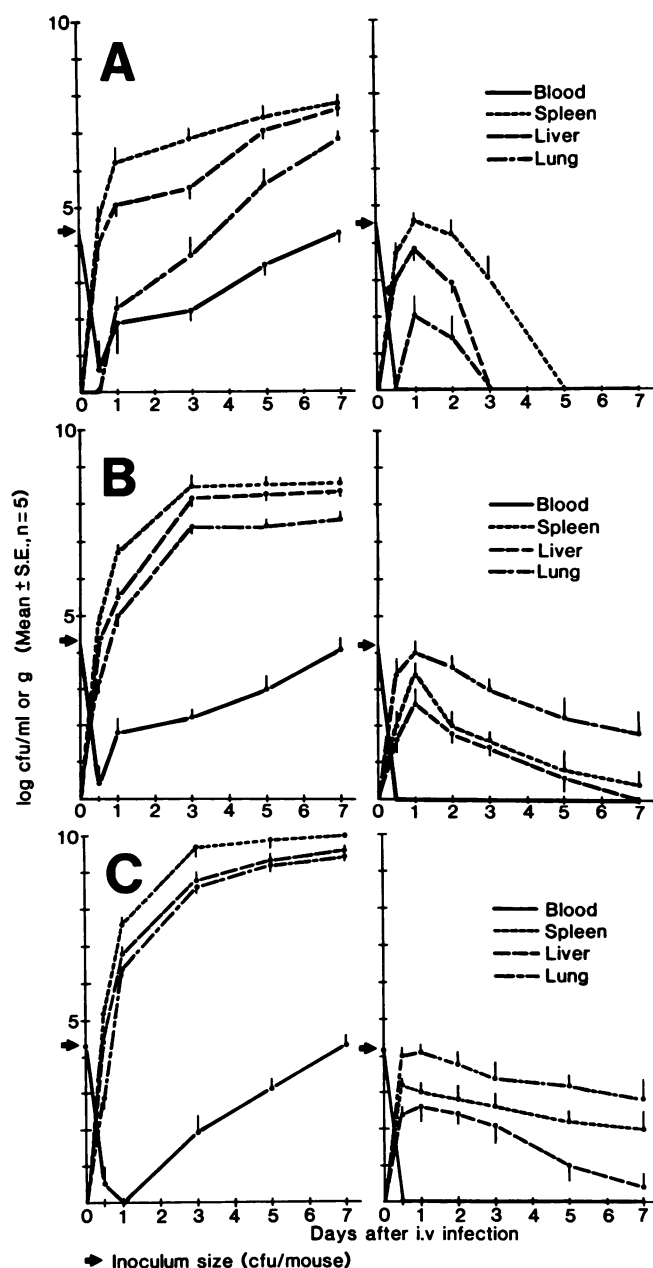


FIG. 1. Recovery from mouse blood and liver after intravenous injection of  $10^4$  bacteria of  $Lcr^+$  (left) or  $Lcr^-$  (right) *Y. pestis* KIM (A), *Y. pseudotuberculosis* PB1 (B), and *Y. enterocolitica* WA (C).

expression of an exotoxin (described below) not present in the enteropathogenic yersiniae (147, 151).

#### Pathology

Little is known about the early events in the experimental infection described above that promote transfer of intravenously injected yersiniae from the vascular system to visceral tissue. However, results obtained from sectioned tissue samples demonstrated that the first detectable pathological manifestation, regardless of species, was appearance of focal necrosis within organs. Mice injected with *Y. pestis* failed to mount a significant inflammatory reaction in re-

sponse to these lesions, which progressively enlarged and eventually coalesced, thus involving whole organs and local vascular systems (Fig. 2A). A similar but less severe attack was mounted by the enteropathogenic yersiniae, which caused formation of more typical abscesses composed of multiplying bacteria and recruited neutrophils (147, 153).

The nature of these lesions was subsequently examined by electron microscopy (135). Results showed that cells of *Y. pseudotuberculosis* were localized in extracellular sites (e.g., liver sinusoids) often in association with thrombocytes. This interaction may facilitate platelet aggregation, thereby inducing vascular thrombosis. Little or no interaction with professional phagocytes was detected. It is anticipated that similar results will be obtained with *Y. pestis* and *Y. enterocolitica*. The most striking finding of both the histological and electron microscope studies was that virulent yersiniae prevented occurrence of the inflammatory response expected of organisms capable of causing severe tissue necrosis. This result would be expected if the organisms promote suppression of cell-mediated immunity (135, 152).

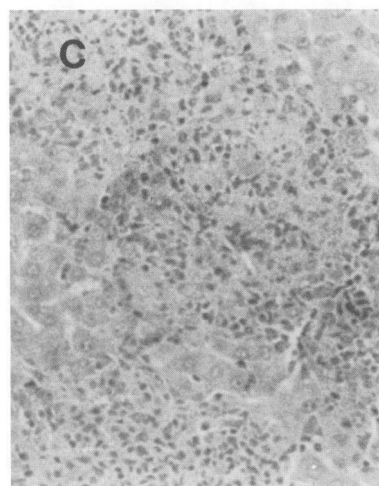
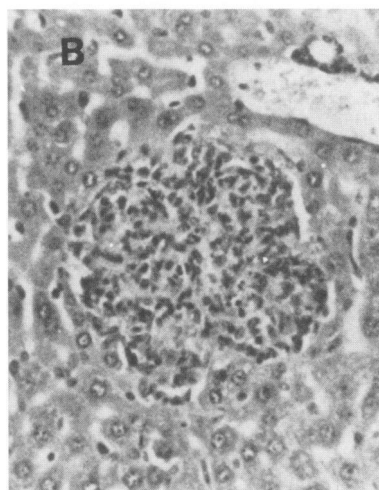
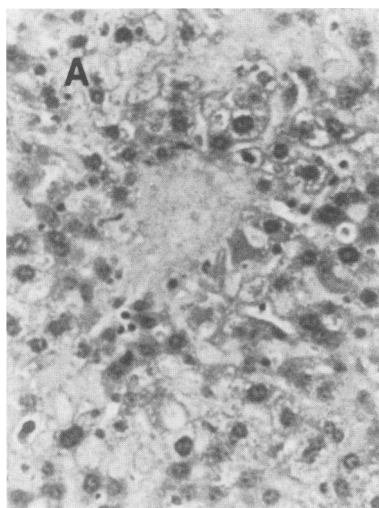
#### Bacterium-Host Cell Interactions

It is established that yersiniae can survive and even grow significantly within a variety of professional phagocytes and their derived lines (39, 41, 67, 83, 115, 148, 149). However, the significance of these observations is obscured by the fact that the experimental work was typically performed in vitro, whereas growth in vivo, as defined in mouse tissue after intravenous injection, is primarily extracellular. Further study may reveal some critical interaction with host cells, involving fixed macrophages monitoring the vascular system, especially during infection following intravenous injection. At present, it is tempting to speculate that the organisms are indifferent to the presence of professional phagocytes, at least in the immunologically naive host. As noted subsequently, this indifference does not apply to certain avirulent mutants that evidently can be phagocytized and killed by neutrophils and monocytes.

Enteropathogenic yersiniae can also promote their own uptake by a variety of nonprofessional phagocytes (61, 80, 163). The nature and distribution of these unique host cell invasins are discussed below. In summary, it is now apparent that yersiniae are primarily extracellular parasites, although the possibility remains that the organisms may undergo critical interactions with both professional and nonprofessional phagocytes. Such interactions, however, would be far less extensive than those described for other injurious facultative intracellular parasites that utilize macrophages as a favored in vivo niche (e.g., brucellae, francisellae, and mycobacteria).

#### PLASMIDS AND HIGH-FREQUENCY DELETIONS

Naturally occurring plasmids are chromosomally independent hereditary entities that provide a bacterial host with some sort of selective advantage in natural environments. As such, not all commensal or saprophytic procaryotes of the same species possess a given plasmid (54). The situation may be distinct in the case of parasites when the plasmid in question encodes factors essential for expression of virulence. Plasmids encoding this type of information are typically present in all wild-type isolates of a given pathogen because their spontaneous cure results in avirulence (thereby relegating the bacterium to competing with saprophytes



in hostile natural environments). Examples include virulence plasmids of *B. anthracis* (69, 99), shigellae (94), and the three elements described in yersiniae (8, 59). The latter consist of a ca. 10-kb pesticin or Pst plasmid, a ca. 70-kb low-calcium-response or Lcr plasmid, and a ca. 100-kb exotoxin or Tox plasmid. The Lcr plasmid is shared by all three yersiniae, whereas only *Y. pestis* harbors the Pst and Tox plasmids. Phenotypic determinants encoded by these elements are described below and listed in Table 1.

### Low Calcium Response

The low calcium response, mediated by the Lcr plasmid, describes the consequences of failure to provide yersiniae with at least 2.5 mM  $\text{Ca}^{2+}$  during cultivation in vitro at 37°C. In this case, cells of *Y. pestis* undergo an ordered nutritional stepdown resulting in cessation of stable RNA synthesis, reduction of adenylate energy charge, and shutoff of cell division (40, 165). However, while in this restricted state, the bacteria initiate synthesis of established and putative virulence factors encoded on the Lcr plasmid including *Yersinia* outer membrane peptides (Yops) and soluble V antigen (14, 17, 27, 90, 107, 143, 144, 164). In addition, they continue production of virulence determinants but not bulk vegetative protein encoded on the chromosome and other plasmids (96). Reduction of temperature to 26°C or addition of 2.5 mM  $\text{Ca}^{2+}$  to restricted organisms results in repression of Lcr plasmid-encoded virulence functions and permits subsequent resumption of vegetative growth (165). Strictness of the low calcium response is potentiated by  $\text{Mg}^{2+}$  (20 mM) and decreased by addition of sufficient levels of nucleotides to assure chelation of  $\text{Mg}^{2+}$  (164) or by removal of  $\text{Na}^+$  (64). Lcr plasmids of the three species of yersiniae share close homology (122). Nevertheless, shutoff of cell division due to  $\text{Ca}^{2+}$  privation is more pronounced in *Y. pestis* than in the enteropathogenic yersiniae (37), and this stringency is a constitutive function of the plague Lcr plasmid per se (144).

The workings of the Lcr plasmid are complex, equivocal, and beyond the scope of this review. Key established features are that structural genes for Yops exist in multiple operons scattered throughout the plasmid (14, 17, 107, 143, 144), whereas major regulatory functions are encoded within a ca. 18-kb sequence termed the  $\text{Ca}^{2+}$  dependence region (14, 45, 46, 68, 118, 121, 155, 161, 162). The latter includes at least five linked, thermally inducible *lcr* or *vir* genes that mediate the expression of Yops and V antigen (17, 45, 62, 63, 68, 113, 124, 155, 161, 162). Loss of these loci usually (17, 45, 68) but not always (162) mimics cure of the Lcr plasmid in that the resulting mutants can grow at 37°C without  $\text{Ca}^{2+}$ , fail to synthesize significant Yops and V antigen, and are avirulent. The *lcrF* locus was assigned a major role in the coordinate thermal induction of virulence determinants (44). An *lcrGVH* operon adjacent to the  $\text{Ca}^{2+}$  dependence region (113, 124) and its attendant linked regulatory *lcrR* locus (5) were also shown to ameliorate  $\text{Ca}^{2+}$ - and nucleotide-mediated regulation of the low calcium response. An important consequence of this line of study was the discovery that a nonpolar *lcrV* mutant (solely unable to express its product V

FIG. 2. Histopathological changes in mouse liver after infection with *Y. pestis* KIM: (A)  $\text{Lcr}^+$  cells exhibiting focal necrosis at day 3 postinfection ( $\times 344$ ), (B)  $\text{Lcr}^-$  cells exhibiting granulomatous lesion with monocytes at day 3 postinfection ( $\times 344$ ), and (C)  $\text{Lcr}^+$  cells exhibiting typical granuloma on day 9 postinfection after passive immunization with polyclonal anti-V ( $\times 172$ ) (108).

antigen) lost nutritional dependence on  $\text{Ca}^{2+}$  at  $37^\circ\text{C}$  but retained ability to express detectable levels of Yops in media lacking the cation (123). This observation indicates that accumulation of an efficacious concentration of V antigen is, in itself, sufficient to promote restriction of cell division.

Working models, based on the findings referenced above, have been proposed that account for much of the complexity of the low calcium response (5, 11, 44, 62, 123). Difficulties encountered in unraveling this phenomenon have stemmed in part from inadvertent use of unstable insertion or polar mutants, ignorance of physiological roles of gene products, and uncertainty of the nature of contributions made by chromosomal genes. With the realization that restricted growth reflects expression of V, future progress in resolving the regulatory cascades mediated by the Lcr plasmid should be rapid.

### Pesticinogeny

The Pst plasmid is unique to *Y. pestis*. It accounts for the singular ability of this species to undergo dissemination from peripheral sites of infection (24) and, as noted below, it accentuates the impact of Lcr plasmid-mediated functions in promoting acute disease (108). This plasmid encodes its own maintenance functions; the bacteriocin, pesticin; a putative pesticin immunity protein; and a plasminogen activator (139, 140). Prolonged cultivation at  $5^\circ\text{C}$  facilitates selective cure of this element (130).

### Exotoxicity and Encapsulation

Until recently, the second *Y. pestis*-specific plasmid had received little attention and was viewed as cryptic in function. Plague exotoxin and capsular antigen are now known to be encoded on this element (125), which has thus been termed the Tox plasmid. In view of its large size (ca. 100 kb), the possibility exists that it may encode additional functions involved in expression of virulence.

### High-Frequency Deletions

The mutation to  $\text{Ca}^{2+}$  independence in *Y. pestis* occurs at the rate of  $10^{-4}$  (75) and was initially assumed to reflect spontaneous cure of the Lcr plasmid. However, further study revealed that up to half of tested Lcr<sup>-</sup> mutants retained an altered Lcr plasmid possessing assorted deletions (or additions) within the  $\text{Ca}^{2+}$  dependency region (119). Occurrence of this phenomenon has not been reported in *Y. pseudotuberculosis* or *Y. enterocolitica*, suggesting that *Y. pestis* may harbor a unique insertion sequence or transposable element. Further evidence favoring this possibility is the widespread general observation of recombination between the plasmids unique to *Y. pestis* and the chromosome (59, 125, 167).

The most dramatic example of high-frequency deletion in *Y. pestis* is the spontaneous mutation from pigmentation (Pgm<sup>+</sup>) to nonpigmentation known to occur at the rate of  $10^{-5}$  (23). This event reflects loss of a chromosomal segment of at least 45 kb (110, 114) but not >189 kb (as judged by results obtained by pulsed-field gel electrophoresis) (21). This sequence encodes iron-inducible peptides and an outer membrane hemin absorption site defined below. Again, this high-frequency deletion does not occur in the enteropathogenic yersiniae which can share the iron-inducible peptides of *Y. pestis* (33–35) but fail to accumulate corresponding levels of hemin.

## ESTABLISHED AND PUTATIVE VIRULENCE FACTORS

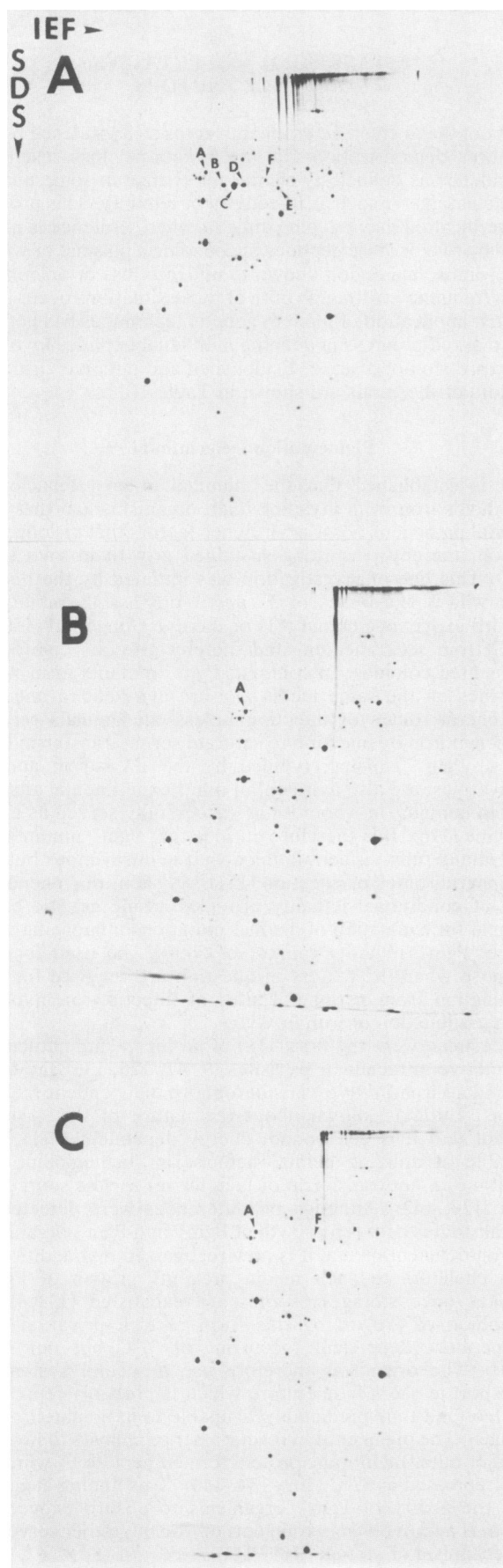
Proof that a given determinant serves as a virulence factor requires demonstration that its mutational loss results in avirulence as defined by significant change in some quantifiable parameter such as infectivity or lethality. This process of verification may become difficult when virulence is mediated by two or more peptides encoded on a plasmid or within a chromosomal region known to undergo loss or deletion at high frequency. Although both of these situations occur in *Y. pestis*, application of modern genetic techniques has permitted marked progress in defining individual peptides involved in expression of disease. Established and putative virulence factors of the genus are shown in Table 1.

### Pigmentation Determinant

It is established that the mammalian host tenaciously withholds iron from invading microorganisms and that successful pathogens possess virulence factors that dislodge the cation, thereby permitting sustained growth in vivo (157, 158). This line of investigation was initiated by the finding that wild-type isolates of *Y. pestis* possess the ability to absorb exogenous hemin (81) or the dye Congo red (150) at  $26^\circ\text{C}$  from solid medium and thereby grow as colored or pigmented colonies. In contrast, Pgm<sup>-</sup> mutants form white colonies on the same media and are avirulent in mice by peripheral routes of infection unless the animals receive sufficient iron by injection to saturate serum transferrin (82). These Pgm<sup>-</sup> isolates, typified by the EV strain and its derivatives, are also avirulent in other experimental animals and in humans, in whom it has successfully served as a live vaccine (116). It is therefore curious that Pgm<sup>-</sup> mutants are essentially fully virulent in mice via the intravenous but not peripheral routes of injection (151). As such, this phenomenon of conditional lethality provides a safe genetic background for evaluation of distinct mutations influencing virulence. Pgm<sup>-</sup> mutants cannot, of course, be used for this purpose when the factors in question are required for dissemination from peripheral sites of infection or involved with assimilation of iron in vivo.

Yersiniae were the first (111) of an increasing number of facultative intracellular parasites (9, 47, 126, 136) shown to assimilate iron in vivo via siderophore-independent mechanisms. Little is known about the nature of this process except that it is cell bound, energy dependent (111), and capable of utilizing hemin, hemoexin, hemoglobin, and ferritin (but not transferrin or lactoferrin) as sole sources of iron (134, 142). Although no differences were detected in initial studies between growth of Pgm<sup>+</sup> and Pgm<sup>-</sup> organisms in iron-deficient media, it is now recognized that addition of iron chelators to such media prevents growth of Pgm<sup>-</sup> isolates once storage reserves are exhausted (133, 134). Inhibition of growth by this form of iron privation was temperature dependent (occurring at  $37^\circ\text{C}$  but not  $26^\circ\text{C}$  [133]). The organisms therefore use a second system of transport at room temperature which is probably repressed in vivo (and thus predictably is unable to assimilate iron in tissues). The pigmentation reaction per se appears to involve a major outer membrane protein termed peptide F, which is also repressed at  $37^\circ\text{C}$  (109, 134, 146). This finding suggests that the ability of Pgm<sup>+</sup> organisms to absorb exogenous hemin is not involved in transport of iron but rather serves as a mechanism of storage (109, 111).

A search for other products of genes contained within the



sequence deleted in  $Pgm^-$  mutants revealed the presence of two high-molecular-weight, iron-repressible peptides (33–35). Although these structures were initially believed to function within the outer membrane, they exist at a higher concentration within cytoplasm (21), where they may fulfill a critical role in transport or storage of the cation. Study of highly purified outer membranes of  $Pgm^+$  organisms grown in iron-deficient medium demonstrated the occurrence of five iron-repressible peptides, termed Irps, of significantly smaller size (134). Of these, only Irp A was retained following high-frequency deletion to  $Pgm^-$  (Fig. 3). The high-molecular-weight, iron-repressible peptides are not processed to form Irps as judged by the phenotype of a spontaneous pesticin-resistant mutant possessing an evident point mutation within the sequence normally deleted in  $Pgm^-$  mutants. This isolate retained the ability to express peptide F (and could thus absorb hemin) and the high-molecular-weight proteins but, like typical  $Pgm^-$  mutants, was unable to grow at 37°C in chelated iron-deficient medium and lacked Irps B, C, D, and E (134). These results indicate that at least one of the missing Irps is required for assimilation of chelated iron. They do not, of course, preclude the possibility that the high-molecular-weight, iron-repressible peptides and as yet unidentified structures are also essential for this process.

Regulation of iron transport in many procaryotes is known to be mediated by binding or release of Fur protein from *fur* sequences flanking structural genes encoding iron stress functions (156). Both Fur and *fur* sequences were detected in *yersiniae* (142).

### Pesticinogeny

As noted above, the extent of genetic information that can be encoded on the small Pst plasmid is limited. After it was shown that loss of pesticinogeny typically prevented dissemination from peripheral sites of injection (24), studies were initiated to determine the nature of the salient invasin. It seemed unlikely that the bacteriocin pesticin was in itself involved in tissue invasion, and this assumption was reinforced by discovery that its antibacterial activity resulted from controlled *N*-acetylglucosaminidase-mediated hydrolysis of peptidoglycan (58), a structure not present in eucaryotic cells. The host range of pesticin is narrow and limited to a few atypical strains of *E. coli* (which may present exposed murein) (60), serogroup IA and IB strains of *Y. pseudotuberculosis* (29), primarily serogroup O:8 strains of *Y. enterocolitica* (78, 79), and nonpesticinogenic but  $Pgm^+$  mutants of *Y. pestis* (23, 53). Independent cultures of the latter were treated with pesticin to determine the mutation rate to  $Pgm^-$  (23), and this process was also used to isolate the rare pigmented but Irp-deficient mutant (134) noted in the preceding section. This relationship between pesticin and Irps would be anticipated if one of the latter served as the target for pesticin (a possibility that has ample precedent among the group B colicins which absorb to outer membrane

FIG. 3. Autoradiograms of two-dimensional gels of purified outer membranes of  $Pgm^+ Pst^-$  (A),  $Pgm^- Pst^-$  (B), and  $Pgm^+ Pst^+$  (C) cells of *Y. pestis* KIM grown at 37°C in iron-deficient medium ( $<0.3 \mu M Fe^{3+}$ ). Letters refer to Irps A, B, C, D, and E and pigmentation-specific peptide F (produced in higher abundance during growth at 26°C). IEF, isoelectric focusing; SDS, sodium dodecyl sulfate. Reprinted from reference 134 with permission of the publisher.

iron-repressible peptides often involved in uptake of the cation) (51).

In this case, the same Irp would be expected to exist on the surface of pesticin-sensitive strains of enteropathogenic yersiniae, thereby enabling these organisms to duplicate the mechanism of iron transport used by *Y. pestis* during growth in vivo. This occurrence would provide a basis for the observation that pesticin-sensitive isolates of *Y. enterocolitica* are typically more pathogenic than their resistant counterparts (71). It is probably significant that the previously defined, pigmented but Irp-deficient, pesticin-resistant mutant of *Y. pestis* arose at the same low frequency (ca.  $10^{-9}$ ) as did pesticin-resistant mutants of enteropathogenic yersiniae. The latter, like typical Pgm<sup>-</sup> deletion mutants of *Y. pestis*, were of reduced virulence in mice (151), but attempts to demonstrate an analogous lesion in assimilation of iron were equivocal (133). Improved methods for removal of iron or use of different chelators may resolve the nature of avirulence in pesticin-resistant enteropathogenic yersiniae. In any event, it seemed probable that pesticin itself played no role in pathogenesis whereas its target fulfills a critical role in promoting disease. This assumption was verified by demonstrating that pesticin-specific mutations on the Pst plasmid do not reduce virulence, whereas those preventing expression of the plasminogen activator inhibit invasion of tissues (66).

The plasminogen activator promotes a potent fibrinolytic reaction and also accounts for a coagulase activity which may be limited to rabbit plasma (7, 139, 140). The primary sequence of this outer membrane peptide has been defined (140). It is synthesized with a leader sequence which becomes removed upon insertion as a 37,000-Da monomer which, in turn, undergoes subsequent autodegradation to form a smaller secondary structure of 35,000 Da (140). The latter conversion occurs in about 2 h at 37°C in enriched medium (Fig. 4). It is not yet known whether one or both of these structures promotes formation of plasmin, although the smaller component accumulates as a major outer membrane peptide (97). Presumably, the fibrinolytic activity of this invasin accounts for dissemination of *Y. pestis* in vivo, although, as defined below, it also significantly modulates the Lcr<sup>+</sup> phenotype of this species.

### Exotoxicity and Encapsulation

Capsular or fraction 1 antigen and murine exotoxin, encoded on the ca. 100-kb plasmid, serve to enhance the acute nature of bubonic plague. Fraction 1 is a surface component consisting of a protein linked to polysaccharide (fraction 1A) and the free protein (fraction 1B) complexed to form a series of serologically similar aggregates (3, 65). Mutational loss of this capsule does not decrease lethality in mice but may delay the onset of morbidity; virulence of nonencapsulated mutants is significantly reduced in guinea pigs (29). The components probably function to prevent phagocytosis by neutrophils and monocytes during initial stages of dissemination from peripheral sites of infection.

Less is known about the structure and mode of action of the exotoxin, which is highly lethal for mice and rats but essentially inert in other hosts. The toxin was reported to function as an adrenergic antagonist and exist as a complex polymer of a 12,000-Da subunit (20, 102). Further study will be required to assess the significance of this activity in promoting disease. As already noted, the exotoxin probably accounts for the more rapid death of mice infected with *Y.*

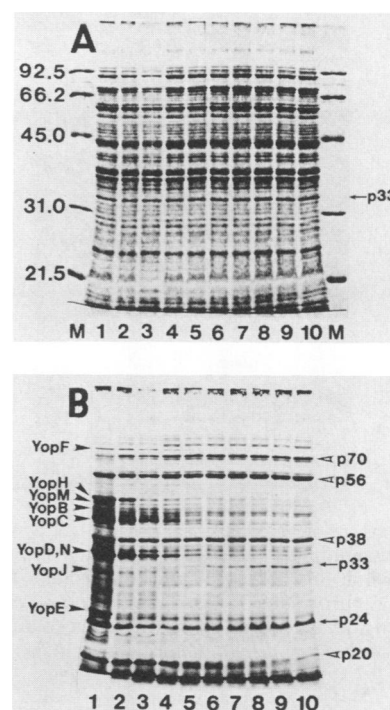


FIG. 4. Stained gel (A) and corresponding autoradiogram (B) of trichloroacetic acid-precipitated material from cultures of Lcr<sup>+</sup> Pst<sup>+</sup> cells of *Y. pestis* KIM after 6 h of growth at 37°C in Ca<sup>2+</sup>-deficient medium (at which time vegetative growth had ceased). Bacteria were pulsed for 1 min with [<sup>35</sup>S]methionine and then immediately precipitated (lane 1) or chased with unlabeled methionine for 5 min (lane 2), 15 min (lane 3), 30 min (lane 4), 1 h (lane 5), 2 h (lane 6), 3 h (lane 7), 4 h (lane 8), 5 h (lane 9), or 6 h (lane 10) before precipitation and preparation for electrophoresis; molecular weight markers (10<sup>3</sup>) are shown in lanes M. Peptides were separated by 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Open arrowheads represent stable peptides known to be produced during the low calcium response (p70 is antigen 5, p56 is GroEL, p38 is V antigen, and p20 is fraction 1), closed arrowheads indicate Yops, and arrows indicate major degradation products (p33 arises after 2 h from plasminogen activator and p24 arises immediately from YopE). Reprinted from *Microbial Pathogenesis* (97) with permission of the publisher.

*pestis* than that occurring after injection with the enteropathogenic yersiniae.

### Low Calcium Response

Mutation to Lcr<sup>-</sup>, characterized by loss of the Lcr plasmid, or the occurrence of a number of point mutations therein results in outright avirulence. In mutants lacking the plasmid, growth in vivo commences as observed for the parent but soon ceases (Fig. 1) as the host mounts a typical inflammatory response characterized by infiltration of neutrophils and monocytes followed by formation of protective granulomas (Fig. 2B) (108, 147, 153).

The nature of the low calcium response is not identical in *Y. pestis* and the enteropathogenic yersiniae despite the fact that the Lcr plasmids of the three species exhibit close DNA relatedness (120, 122) and are functionally interchangeable (43, 113, 144, 159). One difference is that shift of *Y. pestis* to restrictive conditions results in a more abrupt shutoff of cell division than is observed in the enteropathogenic species (37, 112), and this distinction occurs as a function of the Lcr

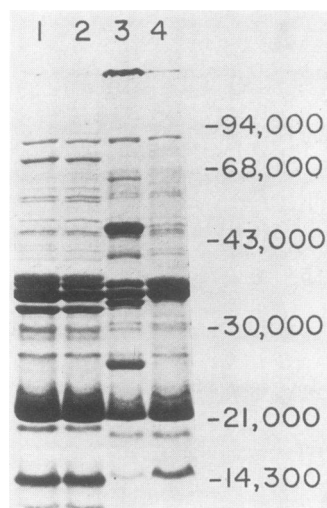


FIG. 5. Comparison of peptides present in a stained gel of purified outer membranes of *Y. pestis* KIM and *Y. pseudotuberculosis* PB1 grown at 37°C in  $\text{Ca}^{2+}$ -deficient medium: Lcr<sup>+</sup> *Y. pestis* (lane 1), Lcr<sup>-</sup> *Y. pestis* (lane 2), Lcr<sup>+</sup> *Y. pseudotuberculosis* (lane 3), and Lcr<sup>-</sup> *Y. pseudotuberculosis* (lane 4). Net accumulation of Yops occurred only in Lcr<sup>+</sup> *Y. pseudotuberculosis* (145).

plasmid itself rather than because of its cytoplasmic background (144). Second, marked qualitative and quantitative differences exist between expression of Lcr plasmid-encoded peptides known or assumed to mediate virulence.

Of these, Yops and the adhesion YadA have received the most attention. With the first comparative determination of these structures (Fig. 5), it became obvious that they were major outer membrane peptides of Lcr<sup>+</sup> *Y. pseudotuberculosis* but were not expressed by *Y. pestis* (145). This observation was puzzling because evident hydrolytic products of Yops produced by *Y. pestis* were found both in vitro and in vivo (15, 95) and the intact peptides were expressed in the cytoplasmic background of *Y. pseudotuberculosis* harboring the Lcr plasmid of *Y. pestis* (144, 159). Results of a search

for the *Y. pestis*-specific factor accounting for the absence of Yops in this species demonstrated that these structures accumulated normally in mutants cured of the Pst plasmid (130). Further work resulted in the finding that Yops were synthesized normally but underwent posttranslational degradation (Fig. 4), resulting in their disappearance within minutes (131); as anticipated, proteolysis was catalyzed by the plasminogen activator (141). Accordingly, Lcr<sup>+</sup> isolates of *Y. pestis* undergo normal synthesis of Yops but promote their almost immediate hydrolytic destruction.

Some properties of major Yops are listed in Table 3. Many of these structures are common to all three yersiniae, whereas others are species specific. Separate mutants lacking structural genes for each Yop have not yet been prepared; thus, it is premature to assume that each functions as a virulence factor. However, mutants unable to produce Yops E (62, 63, 144), H (17), M (90), K, and L (143) are of significantly reduced virulence. The physiological role of Yop E is unclear, although it is established that virulence of *yopE* isolates of *Y. pestis* (following intraperitoneal but not intravenous injection) in mice is phenotypically restored by concomitant injection of iron (97). This observation would be expected if Yop E inhibits one or more of the normal host-mediated antibacterial functions also known to be blocked by injected iron (154). Yop H, known to inhibit phagocytosis (127), was recently reported to possess protein tyrosine phosphatase activity (70), a central regulatory enzyme of eucaryote physiology (104). The significance of this enzyme in pathogenic procaryotes is unknown but undoubtedly ominous (13). Mutants unable to produce Yops K and L were rapidly cleared from organs, suggesting that these structures prevent the onset of cell-mediated immunity (147). Yop M possesses a configuration structurally distinct from but immunologically similar to human platelet surface protein GPIIb and thereby can inhibit aggregation of platelets (90).

Further work on the function of Yops should yield equally interesting findings and bolster the reputation of the Lcr plasmid as a complex but efficient mediator of disease. In addition to defining their mode of action, current effort has focused on establishing the anatomical location of Yops. For

TABLE 3. Properties and distribution of Yops

Determinant	Mol wt (10 <sup>3</sup> )	Produced by:			Essential for full virulence	Remark(s)
		<i>Y. pestis</i>	<i>Y. pseudotuberculosis</i>	<i>Y. enterocolitica</i>		
YadA		0	+	+	0	Adhesion associated with expression of chronic disease
YopB	44	+	+	+		
YopC	42	+	+	+		
YopD	34	+	+	+		
YopE	25	+	+	+	+	Stable degradation product in <i>Y. pestis</i> ; virulence of <i>yopE</i> (i.p.) <sup>a</sup> "restored" by injected iron; required for prompt growth in tissues
YopF	76	+	+			
YopG	58	0	+			
YopH	45	+	+	+	+	Protein tyrosine phosphatase; inhibits phagocytosis
YopI	43	0	+			
YopJ	31	+	+		0	
YopK	22	+	0	0	+	Required for prompt growth in tissues
YopL	16	+	0	0	+	Required for prompt growth in tissues
YopM	45	+	0	0	+	Homologous to platelet glycoprotein Ib; required for prompt growth in tissues
YopN	34	+	+	+		Temp sensor

<sup>a</sup> i.p., intraperitoneally.

example, it was emphasized that these structures lack leader sequences and hydrophobic regions common to typical outer membrane peptides (e.g., the plasminogen activator) and can be detected in spent media (72, 98). Accordingly, Yops may be more appropriately viewed as shed or released peptides (rather than excreted soluble enzymes), and their accumulation at the bacterial surface is artifactual in that they might preferentially adhere to host tissue *in vivo*. Additional evidence favoring the notion that Yops are released through the outer membrane is their destruction in *Y. pestis* by known outer membrane-specific plasminogen activator (97, 130, 141).

Concern regarding anatomical origin does not apply to YadA, an established outer membrane fibrillar structure of enteropathogenic yersiniae (84) known to promote binding to collagen (55). Synthesis of protein 1 is repressed at 26°C but, unlike Yops, is not influenced by concentration of  $\text{Ca}^{2+}$ . This component mediates a plethora of biological effects, including autoagglutination and erythrocyte agglutination (4, 55, 85, 137). It is not expressed in *Y. pestis* due to a frameshift mutation in the Lcr plasmid of this species (128). Its mutational loss in *Y. pseudotuberculosis* enhanced rather than decreased lethality (128). This finding would be anticipated if YadA serves to promote chronic rather than acute disease.

Secreted Lcr plasmid-mediated proteins are scant in number and may be limited to V and W antigens (28, 30, 86). These classical virulence antigens have received considerable attention as possible promoters of  $\text{Ca}^{2+}$  dependence, immunosuppressors, and protective antigens. V antigen itself exists as a ca. 38,000-Da monomer capable of undergoing aggregation upon increase in ionic strength. The protein is unstable in the sense that purified preparations undergo evident autoproteolysis (25). Antiserum raised against W antigen cross-reacted with V, although W is significantly larger (ca. 140,000 Da) and possesses a more acidic isoelectric point (86, 96). GroEL is known to be conserved among procaryotes and to function as a chaperone protein involved in selective secretion (89). An attempt to purify and characterize W antigen resulted in retrieval of homologous GroEL (96). This observation would be expected if W exists as a GroEL-V antigen complex. Unlike Yops, V and W antigens are detected in either cytoplasm or culture supernatant fluid but not in association with outer membranes (130, 145, 146). Additional evidence that V undergoes a specific secretory reaction as opposed to passive release, as evidently occurs with Yops (72, 98), is that V, although vulnerable to hydrolysis via the plasminogen activator, emerges and accumulates extracellularly in an intact form (25).

As noted previously, a clean nonpolar *lcrV* mutant was avirulent, maintained the ability to produce Yops, and lacked a nutritional requirement for  $\text{Ca}^{2+}$  (123). This finding suggests that V itself is bacteriostatic or that the process of its secretion is inconsistent with vegetative growth. If the host was known to possess a niche capable of killing growing but not restricted bacteria (analogous, say, to an *in vitro* environment containing penicillin [160]), then existence of a virulence factor preventing growth in that niche would be understandable. No such *in vivo* environment has been established (but certain nonoxidative killing mechanisms of professional phagocytes are ineffective against resting bacteria [49, 73]). Further work will be required to determine whether avirulence of *lcrV* mutants reflects loss of a regulatory function or a virulence factor *per se* (11, 123).

The instability of V antigen has prevented its direct assay for activities compatible with a role as a virulence factor.

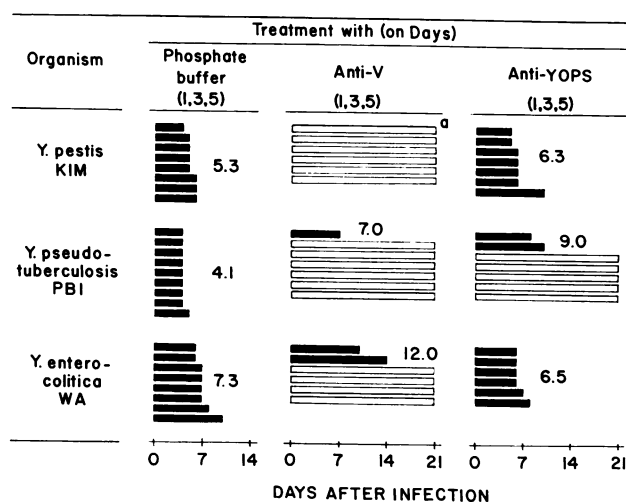


FIG. 6. Ability of rabbit polyclonal monovalent anti-V serum and rabbit polyclonal polyvalent specific anti-Yop serum to provide passive protection in mice against 10 minimum lethal doses of *Y. pestis* (100 bacteria), *Y. pseudotuberculosis* (200 bacteria), and *Y. enterocolitica* (1,500 bacteria). Numbers in parentheses indicate days postinfection on which putative intravenous therapeutic injections (100 µg/mouse) were given. Lengths of solid bars show survival in days of individual mice eventually succumbing to infection; open bars represent survival of individual infected mice until the experiment was terminated at 21 days. Numbers adjacent to solid bars show mean survival times in days (108).

However, crude extracts containing V prolonged survival of Lcr<sup>-</sup> mutants *in vivo*, and this effect was abolished by injection of polyclonal anti-V (153). The latter is known to provide passive protection against infection by both *Y. pestis* (86, 108, 152) and the enteropathogenic yersiniae (108, 152). Analysis of histopathological changes in normal mice and those passively immunized with anti-V demonstrated that the latter formed granulomas surrounding foci of infection by Lcr<sup>+</sup> yersiniae (Fig. 2C) in a manner analogous to those seen in control mice injected with Lcr<sup>-</sup> mutants (153). Similar results were obtained recently with a monoclonal antibody to V antigen (108). As noted previously, Yops K and L were also implicated in maintaining yersiniae in organs (147), suggesting that the process that prevents formation of granulomas is complex and may require a number of Lcr plasmid-encoded activities.

It is perhaps significant in this context that a preparation of anti-Yops (raised against those of *Y. pestis*) provided absolute passive protection against *Y. pseudotuberculosis* but was unable to prevent occurrence of experimental plague (Fig. 6). The significance of this observation is not yet fully understood but possibly reflects the difference in turnover of these Yops by the two species. In this case, anti-Yops would be opsonic to *Y. pseudotuberculosis*, in which they undergo net accumulation, but ineffective against *Y. pestis*, in which they normally undergo degradation mediated by the plasminogen activator. Such constant renewal of the bacterial surface in *Y. pestis* might be expected also to remove other potentially bactericidal molecules involved in host defense and would certainly postpone the emergence of humoral immunity (108). As such, this phenomenon provides another example whereby mechanisms essential for chronic infection are modified in *Y. pestis* to assure expression of acute disease.

### Chromosomal Determinants

All medically significant yersiniae possess functional or cryptic *inv* genes encoding a host cell invasin, permitting efficient penetration of nonprofessional phagocytes (61, 80). In addition, isolates of *Y. enterocolitica* possess a second invasin of lower molecular weight encoded by *ail* (100, 101). An attempt to demonstrate invasion of HeLa cells by *Y. pestis* was not successful, suggesting that *inv* is cryptic in this species (133). However, the possibility remains that the *inv* product is expressed by *Y. pestis* but was phenotypically repressed under the conditions of assay by capsular antigen or some other determinant unique to this species. The role of these host cell invasins in promoting disease is not yet resolved. Since loss of the *inv* product increased virulence of *Y. pseudotuberculosis* (128), it appears that this activity may, like YadA, be required for promotion of chronic infection or needed for prolonged fecal excretion. Perhaps the most interesting aspect of these invasive functions is that they alone can promote translocation of bacteria into host cells, a manipulation that offers obvious possibilities in eucaryotic cell biology. Unable to account for avirulence of pesticin-resistant mutants of enteropathogenic yersiniae by demonstrating a lesion in iron transport (as occurs in Pgm<sup>-</sup> *Y. pestis*), Sikkema and Brubaker (133) proposed that these isolates lacked host cell invasin activity. This correlation is now known to be fallacious (21).

An additional chromosomal determinant of virulence is classical antigen 4 (48) or "pH 6" antigen (12) produced under acidic conditions by *Y. pestis* and *Y. pseudotuberculosis*. Mutational loss of ability to produce this surface component results in autoagglutination (29) and significant loss of virulence (91). It is probably significant that Lcr<sup>+</sup> cells of *Y. pestis* maintain the ability to produce a number of Lcr plasmid-independent virulence factors following Ca<sup>2+</sup> starvation and cessation of vegetative growth (96). Determinants of this group found to abrogate the restriction of transcription imposed during the low calcium response were exotoxin, fraction I, plasminogen activator, and pH 6 antigen. In addition, restricted yersiniae produced a significant concentration of GroEL and a unique catalase probably identical to classical antigen 5 (48). As already noted, GroEL was implicated as a constituent of W antigen. The possibility exists that the novel catalase is also necessary for expression of disease because all other major peptides synthesized during Ca<sup>2+</sup> privation either function as virulence factors or otherwise mediate these factors.

### CONCLUSIONS

The intent of this review has been to emphasize that the Lcr plasmid serves a basic role in all medically significant yersiniae by directly or indirectly promoting a form of immunosuppression that permits unrestrained growth with accompanying necrosis. Superimposed on this effect are antiphagocytic, tissue-invasive, and exotoxigenic activities, mediated by the unique plasmids of *Y. pestis*, which function together to cause acute disease manifested as bubonic plague. In contrast, the enteropathogenic yersiniae superimpose adhesin and host cell invasin activities, which serve to favor occurrence of chronic disease. By prolonging host survival, these robust organisms facilitate their dissemination in natural environments, thereby favoring transmission to new hosts. In contrast, it is necessary that the more fastidious *Y. pestis* promotes lethal disease to assure its transmission to new hosts via fleabite.

### ACKNOWLEDGMENTS

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